Original Article

Primary Drug Resistance to Antituberculous drugs in NWFP Pakistan

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Abstract

Objective: To assess the prevalence of Primary drug resistance to Antituberculous drugs in NWFP

Method: A cross-sectional prevalence study was undertaken to evaluate the prevalence of drug resistance among new TB patients, using a non-probability convenience sampling methodology. Sample size was calculated according to the population and WHO's estimated incidence of smear positive tuberculosis in the province/country. Sputum samples were obtained from 122 newly diagnosed patients of pulmonary tuberculosis from centres in Peshawar and Abbotabad in NWFP.

Results: Sensitivities were performed by proportion method which showed the following resistance values in 118 eligible patients: 15 (12.7 %) samples showed primary resistance to one or more drugs. 8 (6.4%) isolates were resistant to a single drug, 2 (1.6%) were resistant to 2 drugs, 4 (3.2%) to 3 drugs, 1 (0.8%) to 4 drugs while none to all 5 first line agents. Resistance to Streptomycin (10µg/ml) was seen in 7 (5.9%), Isoniazid (1µg/ml) in 10 (8.4%), Rifampicin (5µg/ml) in 3 (2.5%), Ethambutol (10µg/ml) in 2 (1.6%) and Pyrazinamide in 6 (5,0%) samples. Primary Multidrug resistance was 2.5%.

Conclusion: This study suggests that prevalence of MDR amongst untreated patients in NWFP is 2.5%, which is a cause of concern and should be addressed through effective TB control programmes with DOTS strategy (JPMA 58:437;2008).

Introduction

Tuberculosis is a serious public health problem in the developing countries. Worldwide emergence of multidrug resistance tuberculosis (MDRTB) has been reported in both developed and the developing countries and poses a major threat to the control of TB.[1] Drug resistance is primary when it develops in a person who has never received anti TB treatment in the past. This is in contrast to acquired drug resistance, which is present in previously treated patients with inadequate or irregular chemotherapy. World Health Organization-International Union against Tuberculosis and Lung Disease from a global surveillance for antituberculosis-drug resistance, reported the prevalence of Primary MDR at 1.4% and 13% in previously treated patients.[2] A survey from 48 geographic sites revealed that drug resistant tuberculosis is ubiquitous and median prevalence of primary resistance to at least one drug in around 10.7 % and that of Primary MDR only 1%.[3]

In Pakistan, the incidence of TB is estimated to be 181 per 1,00,000 population and each year at least 286,000 new TB cases are added to the existent patient population of around 1.8million.[4] In NWFP alone 36000 new cases develop tuberculosis annually. The level of drug resistance is known to provide an epidemiological indicator to assess the extent of resistant bacterial transmission in the community as well as success or otherwise of National Tuberculosis Programme (NTP). High levels of resistance have been reported in certain regions of the world particularly in Asia and parts of Africa.[5-11] The recommendation to use drug susceptibility tests for monitoring and guiding tuberculosis treatment programme was made many years ago.[12] In view of the practical difficulties in collecting comparable data, the World Health Organization (WHO) proposed a programme of global surveillance of drug resistance in Tuberculosis through its collaborating centres for bacteriology of tuberculosis which would function as supranational Reference Laboratories (SRL) for the respective regions. The proposed programme was based on random sampling of patients reporting to clinics for tuberculosis treatment. Susceptibility testing was to be performed by the reference laboratories based on a common protocol including uniform laboratory methods. As a first step, a regional survey was carried out in 10 Latin American countries.[13] The overall experience gained in Latin America suggested that a sample survey of drug resistance with large failure rates of more than 5% may indicate inadequate routine treatment and high levels of initial resistance, which made survey of drug resistance a priority.[13]

Several countries in Asia and Africa undertook national surveys in accordance with the protocol. Countries including Tanzania,[14] South Africa[15] and India[16] established systematic national surveillance programmes.

In Pakistan no such national/ provincial level survey has ever been carried out. In a country ranked 6th in the world in terms of TB disease burden and with 45% of TB

disease burden of EMRO region, a resistance surveillance study is badly needed in order to determine the prevalence, pattern and trends of anti TB drug resistance in the country. The fact that DOTs strategy has not been implemented in the country until recently and TB patients by and large have been treated unsupervised, it was assumed that primary drug resistance is likely to be high in Pakistan. This hypothesis was supported by reports from different cities of Pakistan pointing towards a high drug resistance rate, One study showed resistance to Rifampicin ((R)) and Isoniazid (INH) to 15% and 11% respectively[17] and in another one resistance to H 25%, R 15%, E 12% and S 12%.[18]

A study from NWFP way back in 1994 also showed relatively high primary and acquired drug resistance.[19] In order to test this hypothesis a study was conducted on patients from all over the province presenting to diagnostic centre in the main cities of NWFP i.e Abbottabad and Peshawar. The objective of this study was to assess the prevalence of primary drug resistance in the province.

Methods

The study was approved by Research and Ethics Committee of Postgraduate Medical Institute Peshawar Pakistan.

The study was designed to determine resistance of Mycobacterium tuberculosis isolates from sputum cultures of newly diagnosed smear positive TB patients, of NWFP Province presenting with features of TB to diagnostic centres in Peshawar and Abbottabad. The Centres were located in the two main cities of NWFP where patients from all over the province report. These included TB control Programme centres in Peshawar and Abbottabad, out patient departments of Govt: Leady Reading Hospital Peshawar and Ayub Teaching Hospital Abbottabad and private clinics of all the investigators where patients from all over the province present themselves or are referrals for consultation. In all a total of 7 centres participated in the study.

Subjects Sputum smear examination was performed at the respective diagnostic centres where the patients suspected to have TB were screened. The subjects fulfilling inclusion criteria with smear positive specimens were enrolled in the study and their sputum were sent to the collection centre of the central laboratory for culture and sensitivity testing.

This study was basically a cross-sectional study, evaluating the prevalence of drug resistance among TB patients diagnosed for the first time, with no prior exposure to TB drugs, using a non-probability convenience sampling methodology. The sample size was calculated according to the population and WHO's estimated incidence of smear positive tuberculosis in the province/country. According to these calculations, a sample size of at least 122 was needed for the study which was collected over a period of 4 months. A sputum specimen in the container provided by the central laboratory along with a form was filled by the investigator at the diagnostic centre, giving beside other details a declaration by the investigator that he has confirmed that the patient has never taken anti-TB drugs in the past. Patients were given an information leaflet and informed consent was taken from all patients.

New Smear Positive Pulmonary T.B patients of any sex and age, living in NWFP with no prior T.B medication history were included. Sputum has collected for Culture before initiation of treatment.

The Department of Microbiology laboratory at Aga Khan University Hospital was used as the Central Laboratory for culture and sensitivity testing. Smears for microscopy were screened using Auramine Rhodamine staining. Positive slides were further confirmed by staining with Kinyoun modification of Ziehl Neelson stain.

Mycobacterial cultures were performed on both liquid as well as solid media. Sediments were cultured at 37^{o}C using Lowenstein Jensen (LJ) medium and MIGIT (Becton Dickinson Diagnostic Instruments Systems). For LJ slant 0.1 ml of concentrated specimen was inoculated and incubated for 8 weeks. MGIT vials were inoculated with 0.5 ml of specimen and incubated at 37^{o}C after supplementation of medium with OADC and PANTA; containing Polymyxin B, Amphotericin B, Nalidixic acid, Trimethoprim and Azlocilin. Growth from the positive LJ slant, and MGIT vials were first stained with Kinyoun and M. tuberculosis was identified by BACTEC NAP TB differentiation test (Becton Dickinson, USA).

Susceptibility testing was performed using standard agar proportion method on enriched Middle brook 7H10 medium (BBL) at the following final drug concentrations; rifampicin 5ug/ml, isoniazid 1ug/ml, streptomycin10ug/ml and ethambutol10ug/ml.[20,21] Disc elusion sensitivity plates were prepared using paper sensitivity disc (BBL). McFarland No. 1 standard suspension of isolate was made from growth on LJ slant and diluted to 10^{-2} and 10^{-4} dilutions. The inoculated plates were incubated at 35^{o}C and examined for growth each week for 8 weeks. M.tuberculosis was considered resistant to a given drug when growth =1% above the antibiotic free control was observed in drug containing area. Pyrazinamide sensitivity was carried out g/ml (BACTEC using the BACTEC 7H12 medium pH6.0 at 100 TM PZA test medium, Becton Dickinson USA) in accordance with manufacturers instructions. MTB H37Rv was used as control with each batch of susceptibility testing.

Data was analyzed using SPSS version 10. The results are presented in the form of tables and graphs.

Results

A total of 122 samples were evaluated of which 119 (97%) were found to be culture positive. Among the culture positive patients, 53 (44.5%) were males and 66 (55.4%) females with M/F ratio 1:1.25. The age range of 75 (63%) patients was between 15-35 years, 33 (27%) were above 35 years of age while 11 (9%) were below the age of 15 years.

On smear examination 11 (9%) out of 119 smear positive specimens collected were found to be smear negative.

Drug susceptibility results were conducted on 119. One sample grew Mycobacterium other than Tuberculosis (MOTT) and hence was excluded from further analysis. Out of the remaining 118 patients, the isolates from 103 (87.3%) patients were fully susceptible to all the 1st line drugs tested, while 15 (12.7%) patients showed resistance to one or more drugs. Resistance to Isoniazid alone or in combination with other drugs was seen in 10 (8.4%) patients. Similarly resistance to streptomycin, Rifampicin, Ethambutal and Pyrazinamide was seen in 7 (5.9%), 3 (2.5%), 2 (1.6) and 6 (5.0) respectively.

Table. Resistance pattern of mycobacterium TB (15 resistant samples).

	Numbers	Percentage
Total Culture +ve	118	100.0
Fully sensitive	103	87.3%
Any Resistance	15	12.7%
Resistance to		
Only H	3	2.5 %
Only R	0	0%
Only E	1	0.8%
Only S	4	3.2%
Only P	0	0%
HE	1	0.8%
HR	0	0%
HP	1	0.8%
HS	0	0%
HSP	2	1.6%
HEP	0	0%
HRP	2	1.6
HRSP	1	0.8%
HREP	0	0%
Any H Resistance	10	8.4%
Any R Resistance	3	2.5%
Any HR Resistance	3	2.5%

Key
H= Isoniazid
R=Rifampicin
E=Ethambutol
S=Streptomycin
P=Pyrazinamide

Resistance to 1 drug was seen in 8 (6.4%) patients, 2 drugs in 2 (1.6%) patients, 3 drugs in 4 (3.2%) patients, 4 drugs in 1 (0.8%) patient and no sample showed resistance to all 5 drugs. Primary MDR was found in 3 (2.5%) patients (Table).

Discussion

The WHO/IUATLD Global Project on Anti-tuberculosis Drug Resistance Surveillance recorded considerable variation in the prevalence of drug resistance among 35 countries in 5 continents. The median prevalence of Primary drug resistance was 9.9% with range of 2% to 41%. Overall, the median prevalence of primary MDR-TB was 1.4% ranging from 0 to 10%. Among the South East Asian Region (SEAR) countries, the prevalence of primary resistance is readily available only for Nepal and Thailand since they participated in the WHO supported Global Project on Anti- tuberculosis Drug Resistance Surveillance in 1994-97. The median prevalence of acquired resistance to any drug was recorded as 23.2% with range of 9.8% to 36.6 %. The median prevalence of primary MDR-TB was 2.5 % significantly higher than the global mean of 1.4%.[22] However such information for other (SEAR) countries, based on standardized protocols and methods, is not available.

Although drugs resistance tuberculosis has frequently been encountered in Pakistan and its presence has been known, there is no comprehensive report, mainly due to limited facilities available, for culture and susceptibility tests across the country. The present study on drug resistance in Pakistan using internationally acceptable guidelines and a standardized methodology gives reliable baseline information.

This study reveals culture positively of 97.6% of all the smear positive patients, confirming the quality of reference laboratory at AKU which was also up to the acceptable standard.

The level of drug resistance to Isoniazid, Rifampicin, and MDR of 8.4%, 2.5%, and 2.5%, respectively in previously untreated cases, as is evident from this study, is not as high as one would have expected, keeping in view that NWFP has recently reached the WHO target of 100% DOTS coverage. These values are comparable with resistance studies in different 3rd world countries.[23-25] However the result of this study is different from other surveys conducted in Pakistan. According to a study by Khan JA, et al, the primary resistance to Isoniazed and Rifampicin was found to be 11% and 15% respectively.[17] n another study resistance was found to be even higher with H 25%, R 15%, E 12% and S19%.[26] This relatively high percentage of resistance to individual drugs in the studies from Pakistan[27] was perhaps due to the faulty selection of patients and it appears that

efforts were not made to separate primary drug resistance from initial or acquired resistance. In view of the above mentioned studies, it was realized that there is a strong need to evaluate the primary resistance to Anti-tuberculosis drugs in a Pan Pakistan study.

There has perhaps been a gradual increase in primary drug resistance over the years. This could be overcome by a strong control programme with DOTS strategy which can reduce the emergence of drug resistance in the community. Since no newer drugs for tuberculosis are likely to become available in the near future, the only options left for the prevention of drugs resistance are effective case finding, prompt and correct diagnosis and successful treatment of patients. Apart from a strong control programme, continuous surveillance of drug resistance will provide information which will serve as a useful parameter in the evaluation of control programmes.

Conclusion

A poorly functioning programme can create MDR-TB much faster than it can be treated, even if unlimited resources are available. There is no single prescription for controlling MDR-TB but the various tools available should be applied wisely. Adoption of DOTS to prevent the generation of resistant strains and careful introduction of second-line drugs to treat patients with MDR are the top priorities for proper control/containment of MDR-TB.

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